

Short Communication

Abnormal Accumulation of Prion Protein mRNA In Muscle Fibers of Patients with Sporadic Inclusion-Body Myositis and Hereditary Inclusion-Body Myopathy

Eva Sarkozi, Valerie Askanas, and
W. King Engel

*From the USC Neuromuscular Center, Department of
Neurology, University of Southern California School of
Medicine, Los Angeles, California*

Sporadic inclusion-body myositis is the most common progressive muscle disease of older patients. The muscle biopsy demonstrates mononuclear cell inflammation and vacuolated muscle fibers containing paired helical filaments and 6 to 10-nm fibrils, both resembling those of Alzheimer brain, and Congo-red positivity. Hereditary inclusion-body myopathy designates patients cytopathologically similar but without inflammation. In both muscle diseases, prion, and several proteins characteristic of Alzheimer brain—eg, β -amyloid protein and hyperphosphorylated tau (which normally are expressed mainly in neurons), and apolipoprotein E—are abnormally accumulated in vacuolated muscle fibers, by unknown mechanisms. We now demonstrate in both muscle diseases that prion mRNA is strongly expressed in the vacuolated muscle fibers, which suggests that their accumulated prion protein results, at least partly, from increased gene expression. This, to our knowledge, is the first demonstration of abnormally increased prion mRNA in human disease. Another novel finding is the increased prion mRNA in human muscle macrophages, and both increased prion protein and prion mRNA in regenerating muscle fibers. The latter indicates that prion may play a role in human muscle development. (Am J Pathol 1994, 145:1280–1284)

Cellular prion protein (PrP^c), expressed mainly in neurons, is a 33 to 37-kd membrane protein anchored by a glycoposphoinositol.^{1–3} Its function is uncertain. In human muscle, its concentration at neuromuscular junctions⁴ supports a hypothesized role in cell-cell recognition, adhesion, or communication.^{5,6} Human PrP^c is encoded by a single gene consisting of two exons and one intron on the short arm of chromosome 20.⁷

Muscle biopsies of patients with sporadic inclusion-body myositis (s-IBM) have muscle fibers with vacuoles containing red and gray staining materials with the Engel-Gomori trichrome reaction,⁸ atrophic muscle fibers, and various degrees of inflammation. By electron microscopy, s-IBM vacuolated muscle fibers contain: 1) cytoplasmic “twisted tubulofilaments” of 15 to 21 nm external diameter that are, in fact, “paired helical filaments (PHFs)”;⁹ 2) 6–10 nm diameter fibrils; 3) membranous material; and 4) amorphous material (reviewed in ref. 9). The PHFs of IBM strikingly resemble PHFs of Alzheimer disease brain.¹⁰

Muscle biopsies from patients with autosomal recessive or dominant hereditary inclusion-body myopathy (h-IBM) have those same muscle fiber changes but lack inflammation in the biopsy.⁹

The exceptional feature of s- and h-IBM vacuolated muscle fibers is abnormal accumulation of a group of proteins: Alzheimer β -amyloid protein (A β),¹¹ C- and N-terminal epitopes of β -amyloid precursor protein (β APP),¹² prion,¹³ apolipoprotein E (ApoE),¹⁴ and ubiquitin (Ub).¹⁵ Like Alzheimer PHFs, IBM PHFs con-

Supported by the Ron Stever and Robert Jani Research Funds.

Accepted for publication August 12, 1994.

Address reprint requests to Dr. Valerie Askanas, USC Neuromuscular Center, 637 South Lucas Ave., Los Angeles, CA 90017-1912.

tain hyperphosphorylated tau.¹⁰ Also localized to IBM PHFs are prion, ApoE, and ubiquitin, but not A β or epitopes of β APP.^{9,13-15} On the 6 to 10-nm amyloid-like fibrils, prion, A β and ubiquitin are co-localized.^{12,13} Moreover, IBM vacuolated muscle fibers contain congophilic intracellular amyloid¹⁶ of uncertain chemical composition. To ascertain whether accumulation of prion protein in IBM vacuolated muscle fibers reflects its increased generation there, we performed *in situ* hybridization using a PrP riboprobe transcribed from a cDNA of the human prion protein gene¹⁷ and localized immunoreactive prion protein on parallel sections.

Materials and Methods

Patients

We studied diagnostic muscle biopsies, performed with informed consent, of 31 patients, aged 34 to 79 years, with the following diagnoses: s-IBM, 10; autosomal-recessive h-IBM, 4 (from 4 different families); polymyositis, 6; morphologically nonspecific myopathy, 2; amyotrophic lateral sclerosis, 4; normal muscle, 5. Diagnosis of all patients was based on clinical, laboratory, muscle-biopsy 18-reaction histochemistry,¹⁸ and ultrastructural studies. All IBM patients had PHFs by electron microscopy and inclusions positive for prion, A β , two other epitopes of β APP, and ubiquitin.

PrP Riboprobe

We isolated a full-length, human PrPcDNA sequence from pUC8 recombinant clone (HuPrPDNA2)¹⁷ using *Eco*RI digestion. The isolated 2.4-kb DNA fragment (nt 1-2432) was inserted into pGEM-3Z (Promega) downstream to the SP6 promoter using T4 DNA ligase (GIBCO BRL). Both antisense and sense RNA probes were transcribed respectively from *Hind*III and *Nde*I linearized recombinant plasmid, using 30 μ mol/L ³⁵S-UTP (1300 Ci/mmol/L) and 10 mmol/L each of unlabeled ATP, CTP, and GTP.

In Situ Hybridization

This was performed on 10- μ transverse sections of fresh-frozen muscle biopsies as described previously.¹⁹ In brief, sections were fixed in 4% paraformaldehyde, treated with 0.5% acetic anhydride in 0.1 mol/L triethanolamine (pH 8.0), and prehybridized for 1 hour at 55 C. Hybridization was performed for 3

hours in prehybridization buffer (50% formamide, 0.75 mol/L NaCl, 0.05 mol/L sodium phosphate buffer pH 7.0, 10 mmol/L EDTA, 200 μ g/ml heparin, 5X Denhardt's solution, 25 μ g/ml poly-A, 25 μ g/ml poly-C, and 500 μ g/ml purified tRNA) containing 10% dextran sulfate and [³⁵S]RNA probe (2 \times 10⁸ cpm/ml). After the hybridization, sections were treated with 20 μ g/ml RNase A, washed under high stringency conditions at 60 C for 1 hour, and agitated overnight in 1X SSC. The dehydrated sections were coated with NTB-2 emulsion and exposed for 8 to 10 weeks.

Immunocytochemistry

Immunocytochemical staining was performed on sections serial to those on which *in situ* hybridization was performed, using the peroxidase-antiperoxidase method following the same general procedure as described.^{4,10-13} To immunolocalize PrP, we used polyclonal antiserum R073 against PrP 27-30,²⁰ which was shown to be very specific in our previous studies.^{4,13} Macrophages were identified with monoclonal antibody Ber-MAC 3 (Dako) and regenerating muscle fibers with a monoclonal antibody against desmin (Zymed).²¹

Results

In normal human muscle fibers, accumulated PrP^c mRNA was present at 20 to 30% of the neuromuscular junctions identified by positive acetylcholinesterase reaction (Figure 1, A, B), whereas nonjunctional regions expressed no detectable PrP^c mRNA (Figure 1, A, B).

In s- and h-IBM, approximately 80% of the PrP immunopositive vacuolated muscle fibers expressed very strong PrP^cmRNA signal (Figure 1, C-P). Within those fibers, the strong PrP^cmRNA signal was typically unevenly distributed, commonly in the form of hot spots (Figure 1, C-P), occasionally corresponding to the uneven deposits of PrP immunoreactivity in them (Figure 1, C-P; Figure 2, D-J).

An interesting, but not disease-specific, finding was slightly to moderately increased, evenly distributed PrP^cmRNA and PrP immunoreactivity in small regenerating (desmin-positive²¹) muscle fibers (Figure 2, A-C). These were found in IBM, polymyositis, and other myopathies, suggesting that PrP^c may play a role in normal muscle-fiber development. In all muscle biopsies containing necrotic muscle fibers, including s- and h-IBM, polymyositis, and other myopathies, macrophages expressed very strong

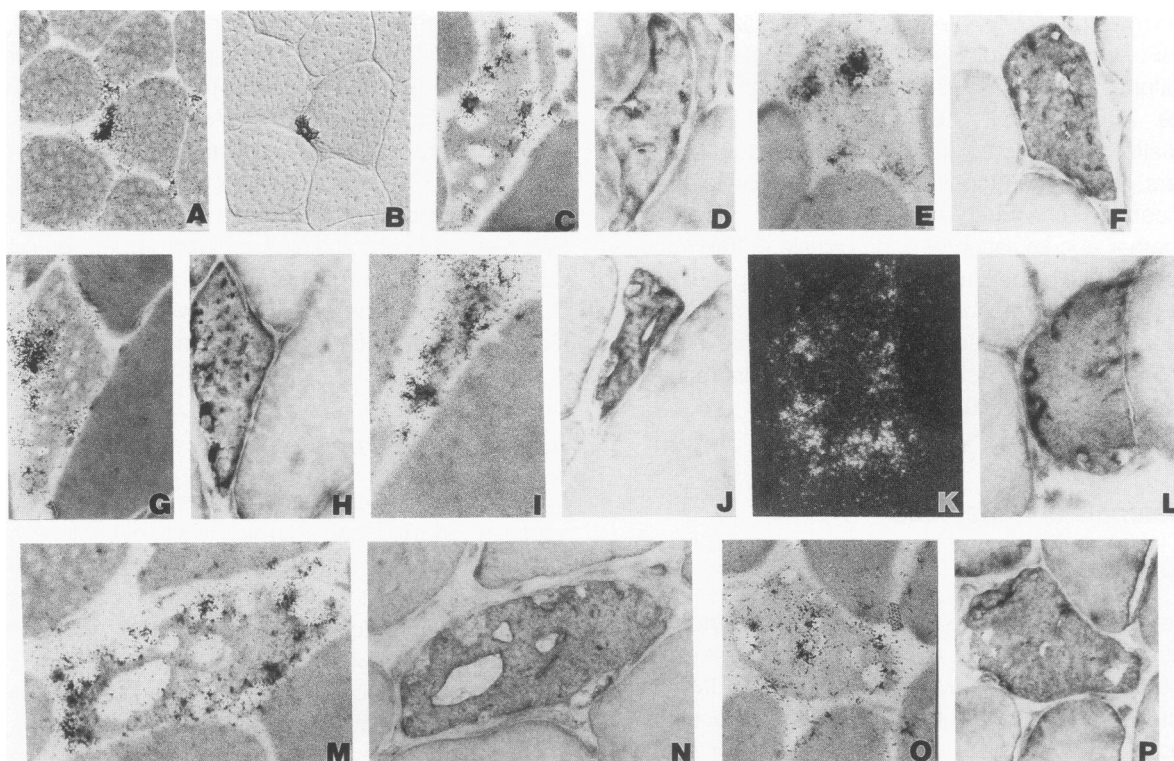


Figure 1. In situ hybridization of PrP^C mRNA in human muscle fibers. In normal fibers, PrP^C mRNA is localized only at the neuromuscular junction (A), where it is co-localized with acetylcholinesterase (B). In sporadic (EF, GH, KL, MN, OP) and hereditary (CD, IJ) IBM, patches of PrP^C mRNA signal (C, E, F, G, I, K, M, O) occur in vacuolated muscle fibers, which also contain immunolocalized PrP protein in multiple foci (D, F, H, J, L, N, P), sometimes corresponding to the PrP^C mRNA patches. Each pair of pictures, A–P, is from parallel (not necessarily adjacent) sections. All photographs are bright-field except B (interference polarization contrast) and K (dark-field). A, B; $\times 700$; C–J: $\times 1240$; K–N: $\times 2400$; O, P: $\times 1240$.

PrP^C mRNA (Figure 2, H–J); however, within the majority of the characteristic IBM vacuolated muscle fibers, mRNA positivity and PrP^C immunoreactivity were not associated with macrophages (Figure 2, D–G).

Sense-strand controls were used throughout and produced only a uniformly weak background signal.

Discussion

This demonstration of increased PrP^C mRNA in IBM vacuolated muscle fibers identifies the first (to our knowledge) human disease manifesting increased PrP^C mRNA. Our study suggests that the PrP abnormally accumulated in IBM muscle results, at least partly, from locally increased transcription of PrP^C .

PrP scrapie (PrP^{Sc}) is a 33- to 37-kd proteinase K-resistant protein resulting from posttranslational conversion of PrP^C protein into PrP^{Sc1-3} , PrP^{Sc} and PrP^C are encoded by the same PrP^C gene.²² That modification of PrP^C can be stimulated by a transmissible agent or an inherited mutation within the

PrP^C gene.¹⁻³ PrP^{Sc} is found in brains of scrapie-infected animals and patients with kuru, Creutzfeldt-Jacob disease, and Gerstmann-Straussler-Scheinker syndrome.¹⁻³ Those brains are characterized by spongiform (vacuolar) degeneration of gray matter, neuronal loss, and prion-positive amyloid plaques.^{1-3,23}

Before our study, the only abnormal human tissue in which PrP and its mRNA have been studied were brains of patients with prion diseases; they did not have increased PrP^C mRNA, nor did brains of scrapie-infected animals.^{1-3,22} Therefore, our findings in IBM contrast with those of prion brain diseases.

Possibly relevant to our results is the recent report that transgenic mice carrying high copy numbers of the wild-type PrP^C gene developed muscle weakness and histological evidence of a non-vacuolar myopathy and a neuropathy.²⁴ Even though that mouse myopathy does not resemble IBM morphologically, it is possible that in IBM muscle the increased expression of the PrP^C gene itself may be pathogenic. Because IBM vacuolated muscle fibers also contain abnormal

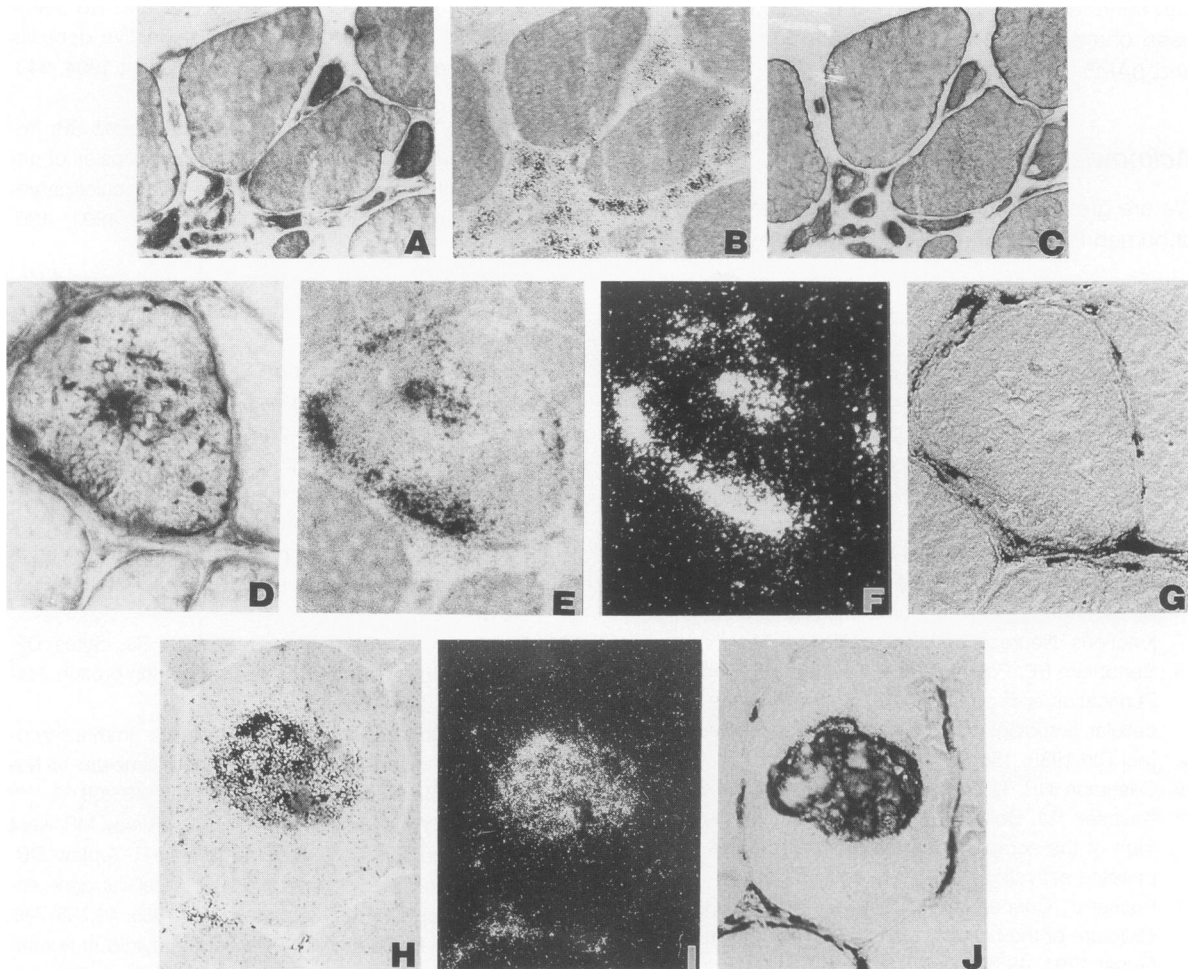


Figure 2. In situ hybridization of PrP^c mRNA in human muscle fibers. In small regenerating muscle fibers (A–C), identified by a monoclonal antibody against desmin (C), there was a slight to moderate amount of diffuse PrP^c mRNA signal (B) and PrP^c protein immunoreactivity (A). Within an IBM vacuolated muscle fiber (D–G), there is strong PrP^c mRNA signal (E, bright-field; F, dark-field) and PrP^c protein immunoreactivity (D), but no immunoreactivity with a macrophage-specific marker (monoclonal antibody Ber-MAC 3) (G). In a polymyositis muscle fiber undergoing phagocytosis (H–J), a cluster of macrophages in the center strongly immunoreactive with the macrophage-specific marker (J) express a strong PrP^c mRNA signal (H, bright-field; I, dark-field). A–C: $\times 1240$; D–J: $\times 2400$.

accumulation of several proteins characteristic of Alzheimer brain and express increased β APP mRNA¹⁹ (mRNAs of the other proteins have not yet been studied), it is possible that one or more of the abnormally accumulated proteins can activate transcription of the other proteins. Because PrP^c and β APP mRNAs can, under certain circumstances, be regulated by the same factor,²⁵ and both β APP²⁶ and prion (this study) are up-regulated in regenerating muscle fibers, an alternative possibility exists. In s- and h-IBM there might be a pathological up-regulation of another, yet-unidentified gene—one normally silent in mature muscle fibers that may, through its product, up-regulate PrP^c , β APP, and other genes normally expressed in brain but not in mature muscle fibers (other

than at neuromuscular junctions). Changes in abnormal muscle fibers of IBM do not simply reflect regenerating properties because they contain PHFs and other IBM-characteristic features not present in regenerating muscle fibers. Therefore, in s- and h-IBM, there may be a co-existing defect in processing or disposal of prion and perhaps some of the other proteins, and/or an abnormal polymerization/binding/precipitation of them (which might or might not be a consequence of supply-side exuberance).

Because the accumulations of PrP and β APP in s- and h-IBM muscle and in abnormal brain have similar features, mechanisms of their accumulation may have important similarities. Cultured IBM muscle, which expresses several aspects of IBM pathology,²⁷

can serve as a living model of a genetic human disease characterized by abnormalities of both prion and β APP.

Acknowledgments

We are grateful to Dr. Stanley B. Prusiner for the gift of human PrPcDNA and PrP antibodies.

References

- Prusiner SB: Molecular biology of prion and diseases. *Science* 1991, 252:1515–1522
- DeArmond SJ, Prusiner SB: The neurochemistry of prion diseases. *J Neurochem* 1993, 61:1589–1601
- Prusiner SB, Hsiao KK: Human prion diseases. *Ann Neurol* 1994, 35:385–395
- Askanas V, Bilak M, Engel WK, Leclerc A, Tomé FMS: Prion protein is strongly immunolocalized at the postsynaptic domain of human normal neuromuscular junctions. *Neurosci Lett* 1993, 159:111–114
- Bendheim PE, Potempska A, Kascsak RJ, Bolton DC: Purification and partial characterization of the normal cellular homologue of the scrapie agent protein. *J Infect Dis* 1988, 158:1198–1208
- Cashman NR, Loertscher R, Nalbantoglu J, Shaw I, Kascsak RJ, Bolton DC, Bendheim PE: Cellular isoform of the scrapie agent protein participates in lymphocyte activation. *Cell* 1990, 61:185–192
- Puckett C, Concannon P, Casey C, Hood L: Genomic structure of the human prion protein gene. *Am J Hum Genet* 1991, 49:320–329
- Engel WK, Cunningham GG: Rapid examination of muscle tissue—an improved trichrome method for fresh-frozen biopsy sections. *Neurology* 1963, 13:919–923
- Askanas V, Engel WK: New advances in inclusion-body myositis. *Current Opinion in Rheumatology*. Edited by Kagen LJ. Philadelphia, Current Science, 1993, pp 732–741
- Askanas V, Engel WK, Bilak M, Alvarez RB, Selkoe DJ: Twisted tubulofilaments of inclusion-body myositis muscle resemble paired helical filaments of Alzheimer brain and contain hyperphosphorylated tau. *Am J Pathol* 1994, 144:177–187
- Askanas V, Engel WK, Alvarez RB: Light- and electronmicroscopic localization of β -amyloid protein in muscle biopsies of patients with inclusion-body myositis. *Am J Pathol* 1992, 141:31–36
- Askanas V, Alvarez RB, Engel WK: β -amyloid precursor epitopes in muscle fibers of inclusion-body myositis. *Ann Neurol* 1993, 34:551–560
- Askanas V, Bilak M, Engel WK, Alvarez RB, Tomé FMS, Leclerc A: Prion protein is abnormally accumulated in inclusion-body myositis. *NeuroReport* 1993, 5:25–28
- Askanas V, Mirabella M, Engel WK, Alvarez RB, Weisgraber K: Apolipoprotein E immunoreactive deposits in inclusion-body muscle diseases. *Lancet* 1994, 343:364–365
- Askanas V, Serdaroglu P, Engel WK, Alvarez RB: Immunolocalization of ubiquitin in muscle biopsies of patients with inclusion-body myositis and oculopharyngeal muscular dystrophy. *Neurosci Lett* 1991, 130:73–76
- Mendell JR, Sahenk Z, Gales T, Paul L: Amyloid filaments in inclusion-body myositis. *Arch Neurol* 1991, 48:1229–1234
- Kretzschmar HA, Stowring LE, Westaway D, Stubblebine WH, Prusiner SB, DeArmond SJ: Molecular cloning of a human prion protein cDNA. *DNA* 1986, 5:315–324
- Engel WK: Muscle biopsies in neuromuscular diseases. *Pediat Clin North Am* 1967, 14:963–996
- Sarkozi E, Askanas V, Johnson SA, Engel WK, Alvarez RB: β -amyloid precursor protein mRNA is increased in inclusion-body myositis muscle. *NeuroReport* 1993, 4:815–818
- Bendheim PE, Barry RA, DeArmond SJ, Stites DP, Prusiner SB: Antibodies to a scrapie prion protein. *Nature* 1984, 310:418–421
- Bornemann A, Askanas V, Engel WK: Immunocytochemical localization of desmin and vimentin in human muscle biopsies. *Neurology* 1990, 40:208
- Oesch B, Westaway D, Walchli M, McKinley MP, Kent SBH, Aebersold R, Barry RA, Tempst P, Teplow DB, Hood L, Prusiner SB, Weissmann C: A cellular gene encodes scrapie PrP 27–30 protein. *Cell* 1985, 40:735–746
- Watanabe R, Duchon LW: Cerebral amyloid in human prion diseases. *Neuropathol Appl Neurobiol* 1993, 19:253–260
- Westaway D, DeArmond SJ, Cayetano-Canlas J, Groth D, Foster D, Yang S-L, Torchia M, Carlson GA, Prusiner SB: Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. *Cell* 1994, 76:117–129
- Mobley WC, Neve RL, Prusiner SB, McKinley MP: Nerve growth factor increases mRNA levels for the prion protein and the β -amyloid precursor protein in developing hamster brain. *Proc Natl Acad Sci USA* 1988, 85:9811–9815
- Sarkozi E, Askanas V, McFerrin J, Johnson SA, Engel WK: Human muscle fibers regenerating in vivo and in vitro express strong β -amyloid precursor protein mRNA while normal adult fibers do not. *Soc Neurosci Abstr* 1993, 19:1314
- Alvarez RB, Fardeau M, Askanas V, Engel WK, McFerrin J, Tomé FMS: Characteristic filamentous inclusions reproduced in cultured innervated muscle fibers from patients with familial “inclusion-body myositis” (FIBM). *J Neurol Sci* 1990, 98:178